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(54) Title: ROLLING CIRCLE AMPLIFICATION ASSAY FOR NUCLEIC ACID ANALYSIS

(57) Abstract: Method and reagents for analysis of nucleic acid sequences is disclosed. In this method a plurality of padlock probes is provided. Each padlock probe may hybridize to a locus on a target nucleic acid under hybridization conditions. If a targeted variant is present at the locus, the padlock probe may be ligated to form an amplification target circle. The amplification target circle acts as a template for production of tandem-sequence DNA. The tandem-sequence DNA may then be digested into non-tandem detection fragments which are subsequently separated and detected. The plurality of padlock probes are designed such that ligation of the probes, amplification of the target circle, and digestion of the tandem-sequence DNA subsequently produced, and detection may all be effected with the same set of reagents. Each probe targets a unique locus variant on the target nucleic acid sequence and produces a detection fragment that may be distinguished from detection fragments produced from other padlock probe in the plurality of padlock probes by using a fragment analysis detector.

INTERNATIONAL SEARCH REPORT

Into all Application No PCT/US 01/03439

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A. CLASSI IPC 7	FICATION OF SUBJECT MATTER C12Q1/68		
	o International Patent Classification (IPC) or to both national classification	ation and IPC	
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Documental	tion searched other than minimum documentation to the extent that s	such documents are included in the	fields searched
Electronic d	ata base consulted during the International search (name of data ba	se and, where practical, search ten	ms used)
EPO-In	ternal, MEDLINE, BIOSIS, WPI Data, F	°AJ	
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the rel	evant passages	Relevant to claim No.
X	LIZARDI P M ET AL: "MUTATION DET AND SINGLE-MOLECULE COUNTING USIN ISOTHERMAL ROLLING-CIRCLE AMPLIFT NATURE GENETICS, NEW YORK, NY, US VOl. 19, no. 3, July 1998 (1998-0	1-11	
	225-232, XP000856939 ISSN: 1061-4036 the whole document	,,,, pages	
Α	WO 00 04193 A (UNIV YALE) 27 January 2000 (2000-01-27) page 15 page 24-25 page 53-58; claims		1-28
A	US 5 854 033 A (LIZARDI PAUL M) 29 December 1998 (1998-12-29) column 1-3; claims; example 5	-/	1-28
X Furt	her documents are listed in the continuation of box C.	χ Patent family members a	ure listed in annex.
"A" docume consider filling of "L" docume which citatio "O" docume other "P" docume."	ent defining the general state of the art which is not dered to be of particular relevance document but published on or after the international date ent which may throw doubts on priority claim(s) or is cited to establish the publication date of another in or other special reason (as specified) sent referring to an oral disclosure, use, exhibition or means ent published prior to the international filing date but han the priority date claimed	"Y" document of particular relevant cannot be considered to involved document is combined with o	Allct with the application but ple or theory underlying the size; the claimed invention or cannot be considered to the size of the document is taken alone size; the claimed invention live an inventive step when the or more other such docunning obvious to a person skilled
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Name and	mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Reuter, U	

INTERNATIONAL SEARCH REPORT

Inti val Application No PCT/US 01/03439

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT Category Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Relevant to claim No.
A LIO DO ADOLE A CINTY DOCUMENTO	
WO 99 09216 A (UNIV ROCHESTER) 25 February 1999 (1999-02-25) page 6-9; figures 1,2 page 34-35	1-28

INTERNATIONAL SEARCH REPORT

Inti 1al Application No PCT/US 01/03439

Patent document cited in search report		Publication date	Patent family member(s)		Publication date	
WO 0004193	Α	27-01-2000	ΕP	1098996	A1	16-05-2001
			WO	0004193	A1	27-01-2000
			US	6316229	B 1	13-11-2001
US 5854033	A	29-12-1998	AT	199572	T	15-03-2001
			AU	714486	B2	06-01-2000
			AU	1024097	Α	11-06-1997
			DE	69612013		12-04-2001
			DE	69612013	T2	02-08-2001
			DK	862656	T3	09-04-2001
			ΕP	0862656	A1	09-09-1998
			JР	2002503948	T	05-02-2002
			WO	9719193	A2	29-05-1997
			US	6183960	B1	06-02-2001
			US	6210884	B1	03-04-2001
			US	6329150	B1	11-12-2001
			US	6344329	B1	05-02-2002
			US	6143495	A	07-11-2000
WO 9909216	Α	25-02-1999	US	6077668	Α	20-06-2000
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		30 -2 2000	ĀŪ	8782198		08-03-1999
			EP	1019535		19-07-2000
			WO	9909216		25-02-1999